Remarks

Claims 123-146 are pending. Claims 123 and 130-135 are amended. The amendments are supported by the application as filed, thus there is no issue of new matter. Claims 125-129 and 146 are cancelled without disclaimer or prejudice to applicants' right to pursue patent protection for the subject matter thereof in another application. Claims 123-124 and 130-145 thus appear in the application.

Support for the amendments is found, *inter alia*, in the specification as follows: **Claim 123**: page 11, line 33 to page 12, line 2, page 12, lines 24-26, page 13, lines 8-10, page 14, lines 1-5 and page 53, line 35 to page 54, line 4; **Claims 130-131**: dependencies changed from claim 129 to claim 123 due to the cancellation of claim 129; **Claim 132**: page 11, line 33 to page 12, line 2, page 12, lines 24-26, page 13, lines 8-10 and page 53, line 35 to page 54, line 4; **Claim 133**: page 12, lines 24-25; **Claim 134**: page 11, line 33 to page 12, line 2, page 12, lines 24-26, page 13, lines 8-10, page 14, lines 1-5 and page 53, line 35 to page 54, line 4; **Claim 135**: page 11, line 33 to page 12, line 2, page 12, lines 24-26, page 13, lines 8-10, page 14, lines 1-5, page 15, line 27 to page 16, line 5, page 53, line 35 to page 54, line 4 and the Experimental Results discussed at pages 36-118.

Applicants note their appreciation for the courtesies extended by Examiner Holleran and her Supervisor, Examiner Anthony Caputa, to their representatives, John P. White, Esq. and Mark A. Farley, Esq. during a telephone interview concerning related application Serial No. 08/196,154 on Tuesday, December 2, 2003. The amendments and comments submitted in this Response are in accordance with the matters discussed during that telephone interview and thus constitute a written record thereof.

REJECTIONS WITHDRAWN

In ¶4 of the Office Action the Examiner stated that the provisional double-patenting rejection of claims 109-122 (and new claims 123-146) over Application No. 08/196,154 is withdrawn because the claims of

copending application No. 08/196,154 are drawn to conjugates comprising a GM2 ganglioside, whereas the claims of the instant application are drawn to conjugates comprising GD2, GD3 lactone, O-acetyl GD3 or GT3.

In ¶5 of the Office Action the Examiner stated that the rejection of claim 95 under 35 U.S.C. 103(a) over Wiegand et al (U.S. Patent 5,599,914), in view of Fiume et al (Critical Rev. Therapeutic Drug Carrier Systems, 4(4): 265-284, 1988) Livingston et al. (Cancer Research, 149:7045-7050, 1989), Ritter et al. (Seminars in Cancer Biology, 2:401-409, 1991), Livingston et al. (U.S. Patent No. 5,102,663), Kensil et al.(The Journal of Immunology, 146(2):431-437, 1991), Marciani et al. (Vaccine, 9:89-96, 1991) and Uemura et al (J Biochem, 79(6):1253-1261, 1976) as applied to claims 78, 80-92, 94 and 96-99 and further in view of Irie (U.S. Patent 4,557,931) is withdrawn upon further consideration of the teachings of Irie with respect to the expression of GD2, GD3 lactone, O-acetyl GD3 or GT3 ganglioside expression in epithelial tissues.

OBJECTIONS/REJECTIONS MAINTAINED

On page 2 of the Office Action dated June 10, 1996 (Paper No. 8) the Examiner stated that the disclosure of the application is objected to since, on page 5, line 30, in the Brief Description of the Figures, Figure 6b is listed as IgG antibodies, but Figure 6b has the y-axis labeled as IgM titer. The Examiner stated that appropriate correction is required. In applicants' response submitted December 13, 1996, applicants stated that they would submit a revised Figure 6B correctly labeling the Y-axis as IgG when this application is in condition for allowance. Applicants accordingly requested that the Examiner withdraw and reconsider the objection to the specification.

Thereafter, in the Office Action dated April 1, 1998 (Paper No. 13) the Examiner stated, in ¶3 on page 2, that Applicants set forth they will submit a new Figure 6B to overcome the rejection when the case is in condition for allowance. The Examiner then stated that until Applicants

submit a proper Figure, the objection is maintained.

Although this ground for objection has not been repeated in subsequent Office Actions, including the present Office Action mailed June 10, 2003, applicants believe that it is still being maintained.

Attached hereto as **Exhibit A** is an annotated (marked-up) copy of Figure 6B indicating in red ink a proposed change wherein the Y-axis is now labeled as "IgG". **Exhibit B** is a replacement drawing sheet with the above-indicated change made to the labeling of the Y-axis. The amendment to Figure 6B is supported by page 5 of applicants' specification. Applicants respectfully request entry of this drawing correction as it raises no issue of new matter. The Examiner is requested to reconsider and withdraw her objection to the disclosure in view of the submission of the corrected Figure.

Double-Patenting Rejections

In ¶6 of the Office Action the Examiner stated that claims 123-146 are provisionally rejected for double-patenting over Application No. 08/475,784. The Examiner stated that the claims of copending application No. 08/475,784 are drawn to conjugates comprising a GD3 ganglioside, and the claims of the instant application are drawn to conjugates comprising GD2, GD3 lactone, O-acetyl GD3 or GT3. The Examiner stated that although the conflicting claims are not identical, they are not patentably distinct from each other because they all claim conjugates and methods of using said conjugates, where the conjugate comprises a ganglioside conjugated to a protein through the ceramide portion. The Examiner stated that it appears that 08/475,784 is drawn to a genus of conjugates that comprise GD3 lactone or O-acetyl GD3, where the genus is small, and GD3 lactone or O-acetyl GD3 are obvious species of the genus GD3. The Examiner stated that therefore, the provisional rejection is maintained. The Examiner stated that applicant argues that the provisional rejection should be allowed to drop and that the instant claims be allowed to issue, pursuant to MPEP section 804. The Examiner stated that since the instant claims are not allowable, the

provisional double patenting rejection is maintained for the reasons of record.

The Examiner additionally stated in ¶7 of the Office Action that claims 123-146 are provisionally rejected for obviousness-type, double patenting over Application No. 08/477,097 for reasons already made of record. The Examiner stated that although the conflicting claims are not identical, they are not patentably distinct from each other because they all claim conjugates and methods of using said conjugates, where the conjugate comprises a ganglioside conjugated to a protein through the ceramide portion. The Examiner stated that thus, the particular species drawn to GD2 claimed in the copending application would anticipate the instant genus composition claims. The Examiner stated that applicants argue that the provisional rejection should be allowed to drop and that the instant claims be allowed to issue, pursuant to MPEP section 804. The Examiner stated that since the instant claims are not allowable, the provisional double patenting rejection is maintained for the reasons of record.

In response to these provisional double-patenting rejections, submitted herewith as **Exhibit C** is a Terminal Disclaimer over any patent issued from U.S. Serial No. 08/475,784 and any patent issued from U.S. Serial No. 08/477,097. The disclaimer has been executed by an authorized representative of Sloan-Kettering Institute for Cancer Research, i.e., the Assignee of the subject application as well as U.S. Serial Nos. 08/475,784 and 08/477,097. As set forth in §804 IB of the Manual of Patent Examining Procedure, the "provisional" double-patenting rejection will become an "actual" double-patenting rejection if either or both U.S. Serial No. 08/475,784 and/or 08/477,097 issue as a patent prior to or on the same date as the present application. The effect of the terminal disclaimer would be to prevent the term of any patent issuing based on the present application from extending beyond the term of any patent based on U.S. Serial No. 08/475,784 or 08/477,097. The Examiner is respectfully requested to reconsider and withdraw the provisional double-patenting rejection of claims 123-146.

Rejections Under 35 U.S.C. §103(a)

In ¶8 of the Office Action claims 123-132 are rejected under 35 U.S.C. 103(a) over the combination of six references, i.e., Wiegand et al (U.S. Patent 5,599,914) in view of Fiume et al (Critical Rev. Therapeutic Drug Carrier Systems, 4(4): 265-284, 1988),Ritter et al. (Seminars in Cancer Biology, 2:401-409, 1991), Kensil et al.(The Journal of Immunology,146(2):431-437,1991), and Marciani et al. (Vaccine, 9:89-96, 1991) and Uemura et al (J Biochem, 79(6):1253-1261,1976).

In ¶9 of the Office Action claims 123, 132-135 and 137-146 are rejected under 35 U.S.C. 103(a) over the combined disclosure of eight references, i.e., Wiegand et al (U.S. Patent 5,599,914), Fiume et al (Critical Rev. Therapeutic Drug Carrier Systems, 4(4): 265-284, 1988) Livingston et al. (Cancer Research, 149:7045-7050, 1989) in view of Ritter et al. (Seminars in Cancer Biology, 2:401-409, 1991), Livingston et al. (U.S. Patent No. 5,102,663), Kensil et al.(The Journal of Immunology, 146(2):431-437, 1991), and Marciani et al. (Vaccine, 9:89-96, 1991) and Uemura et al (J Biochem, 79(6):1253-1261, 1976).

In ¶10 of the Office Action, claims 135 and 136 are rejected under 35 U.S.C. 103(a) over the same eight references as discussed in the paragraph above, combined with two additional references newly cited in this Office Action, namely Diatlovitskaia et al. (Biokhimiia, 56(3): 560-564, 1991, Mar.; Abstract only) and Ritter II (Ritter et al, Immunobiology, 182(1): 32-43, 1990; Abstract only), for a total of ten(10) references. The Examiner stated that Diatlovitskaia teaches that the ganglioside GD3 is expressed in breast carcinomas, which is an example of a cancer of epithelial origin. The Examiner further stated that Ritter II teaches that GD3 derivatives such as GD3 lactone are more immunogenic than GD3.

It is respectfully noted that the first eight references relied upon, in combination, by the Examiner to reject the claims under 35 U.S.C. §103(a) have been discussed in detail in, *inter alia*, applicants' Amendment In Response To December 31, 2002 Office Action filed April 4, 2003 wherein

applicants described several features which they submit distinguish the invention. These arguments are specifically incorporated by reference herein and thus they will not be repeated here. Applicants have now, moreover, amended the claims such that, as discussed below they now recite several additional features which patentably distinguish the invention over the prior art.

The Inclusion Of the Adjuvant QS-21 Provides Unexpected Results That Demonstrate The Non-Obviousness Of The Invention

Claims 124 and 132 were amended to specifically recite that the composition of the invention includes the adjuvant QS-21, i.e., a saponin derivable from the bark of a Quillaja saponaria Molina tree. Claim 133 also includes this material due to its dependence on claim 132. Claims such as 123 and 134-135 recite a saponin derivable from the bark of a Quillaja saponaria Molina tree which, by definition, encompasses the QS-21 adjuvant. During the December 2, 2003 interview, Applicants' representative directed the Examiner's attention to pages 94-95 of the application, which describe unexpected results achieved with compositions as claimed including QS-21 as an adjuvant in comparison to those obtained with corresponding compositions containing prior art adjuvants, i.e., BCG and DETOX.

Briefly, the specification teaches that local reactions to dosages of 100-200 µg of QS-21 were "quite different" (p.94, line 5) than those seen with comparable dosages of the prior art adjuvants BCG and DETOX. It further states that the local response is more diffuse than the response generally seen with doses of DETOX or BCG inducing comparable systemic symptoms (lines 8-11). It additionally teaches (lines 11-16) that a surprising feature of the subjects' response to QS-21 was that several days later (at most 10 days later) the local reactions had completely abated and there was no evidence that the vaccination had been administered to that site.

Applicants' specification additionally teaches (see paragraph bridging pps. 94-95) that QS-21, at any of the dosages used, resulted in a qualitatively different response than responses achieved with the prior art adjuvants to GM2 ganglioside. The results obtained with QS-21 were contrasted against the immunogenic response achieved with the use of GM2-KLH vaccines alone or with optimal doses of BCG or DETOX, which were demonstrated to be substantially less effective than comparable compositions including QS-21. The specification additionally teaches that the results achieved by applicants demonstrate that the 100 and 200 µg doses of QS-21 induce the optimal antibody response against GM2 and that the 100 µg dose is better tolerated. These dosages are specifically recited in applicants' claims.

To summarize, applicants contend that the inclusion of QS-21 in their claimed compositions produces two unexpected improvements over the results achieved with the prior art BCG and DETOX adjuvants: (1) the side effects attributed to such adjuvants are ameliorated with the use of QS-21; and (2) even at the lowest doses of the QS-21 adjuvant, all of patients tested produced IgG antibodies against GM2. Applicants' claims identified above specifically recite the presence of the QS-21 adjuvant and further, that such adjuvant is present in the composition in amount between about 10 µg and about 200 µg (i.e., by virtue of claim 124's dependency from claim 123). Applicants contend that these recitations patentably distinguish the claimed invention from the prior art.

During the December 2, 2003 interview the Examiner inquired whether the above-described results attributable to the inclusion of QS-21 were truly unexpected in light of the disclosure of the Kensil et al. reference. Applicants' representative pointed out that the reference does not suggest the use of QS-21 as an adjuvant and, in fact, teaches away from such use. For instance, page 435 of Kensil et al. makes clear that QS-7 adjuvant is more advantageous than QS-21 in that QS-7 is both less toxic and less hemolytic than QS-21. These advantages of QS-7 over QS-21 are important since the adjuvant is to be combined with the conjugate

(discussed below) for administration to human subjects to stimulate or enhance the production of antibodies and/or to treat a human subject having cancer. Clearly, the increased toxicity and hemolytic activity of QS-21 disclosed in Kensil et al. teaches away from the use of QS-21 and toward the use of QS-7. In summary, Kensil et al. would not lead one of ordinary skill in this art to expect the surprising results achieved using QS-21 as the adjuvant which results demonstrate the non-obviousness of applicants' claimed invention.

The Claimed Conjugate And The Molar Ratio Of Conjugated Ganglioside Derivative To Keyhole Limpet Hemocyanin Provide Additional Evidence Of Patentability

The claims recite, *inter alia,* (1) a conjugate of a ganglioside derivative and Keyhole Limpet Hemocyanin, wherein the ganglioside derivative is a derivative of a ganglioside selected from the group consisting of GM2, GD2, GD3 lactone, O-acetyl GD3 and GT3 and (2) that when the ganglioside is GM2, the GM2:Keyhole Limpet Hemocyanin molar ratio is from 200:1 to 1400:1. These features further distinguish the invention from the prior art relied upon to reject the claims.

The primary reference cited by the Examiner is U.S. Patent No. 5,599,914 to Wiegand et al. ("Wiegand"). Wiegand discloses, e.g., at col. 7, lines 1-8, that the ganglioside derivatives (GM3, GD3, GM2 and GM1) were reacted with Human Serum Albumin, i.e., not Keyhole Limpet Hemocyanin as recited in applicants' claims, which was derivatized with 16-18 SPDP molecules. The reference also teaches that this level was the preferred level (see, e.g., col.7, lines 1-3). In contrast, applicants' claims recite a GM2:Keyhole Limpet Hemocyanin molar ratio of between 200:1 and 1400:1. Such a ratio is neither taught nor suggested by Wiegand. The subject reference teaches away from the invention due to the teaching therein that the derivatization level of 16-18 is the "desired" level, and in view of the use of Human Serum Albumin as the carrier instead of Keyhole Limpet Hemocyanin as recited in applicants' claims. There is no

disclosure in the reference, moreover, which would suggest the replacement of Human Serum Albumin with Keyhole Limpet Hemocyanin, or to produce a conjugate having a derivatization level different than that disclosed in Wiegand.

The Examiner combined Wiegand patent with Fiume et al. ("Fiume"), stating that "Wiegand in combination with Fiume teaches a glycoconjugate as claimed in claims 123 and 132." Applicants respectfully traverse this contention. The portion of Fiume cited by the Examiner (commencing at page 268) states that a **drawback** to the clinical use of the conjugates disclosed therein is their immunogenicity. The thrust is therefore to find a methodology for reducing the immunological effect of these conjugates. This teaching is opposite to that provided by the applicants about their invention in that the purpose of the conjugates of the present invention is to increase, **not** to reduce, the immunogenic effect (see, e.g., claim 134).

Fiume not only teaches away from the present invention, it contains no disclosure which would suggest its combination with Wiegand. Wiegand discloses the formation of a composition for use in producing an immunogenic response. In contrast, Fiume teaches to proceed in a diametrically opposed direction, i.e., to seek compounds having a reduced immunogenic effect. These contrasting teachings would lead a skilled artisan away from combining Wiegand with Fiume. Further, even if combined, such combination would not produce the claimed glycoconjugate.

The Improved Results Obtained With Applicants' Compositions Evidence The Patentability Of These Compositions

Submitted herewith as **Exhibit D** is a copy of a reference by Chapman et al., Clinical Cancer Research, Vol. 6, pp. 874-879 (March 2000) entitled, "Induction of Antibodies Against GM2 Ganglioside By Immunizing Melanoma Patients Using GM2-Keyhole Limpet Hemocyanin + QS21 Vaccine: A Dose-Response Study" (hereinafter "Chapman"). As disclosed in the reference,

in clinical trials melanoma patients vaccinated with GM2-KLH + QS-21 made using the conjugation procedure described in the present application, produced high titer IgM and IgG antibodies specific for GM2. These clinical results led the authors (including Dr. Philip O. Livingston, a co-inventor of the present invention) to state that the GM2-KLH/QS-21 composition, formulated as presently claimed, "is more immunogenic than our previous formulation." (see Abstract). The "previous formulation" referred to in the subject reference involved immunization of subjects with GM2 and *bacilli Calmette-Guerin* (BCG).

The Livingston paper and the Livingston '663 U.S. Patent both disclose the GM2-BCG formulation, i.e., the "previous formulation". The improved formulation described in the present application and claimed herein is distinguishable thereover in that the "previous formulation" does not comprise the same components as the compositions recited in the applicants' claims. Further, not only are applicants' formulations made with different components, the present claims additionally recite specific ranges for the components included in these formulations. As demonstrated in Chapman, the presently claims compositions produce significantly improved results in contrast to those achieved with the previous formulations.

In summary, the claimed compositions are distinguishable over those in the Livingston references as, due to (1) differences in the components from which they are formed, and (2) the relative amounts of the conjugate and the saponin included in these present compositions, as taught by Chapman they produce a substantially improved immune response to subjects in a clinical setting.

Neither the remaining references previously cited by the Examiner, i.e., Ritter I, Marciani et al. and Uemura et al., nor the newly-cited Diatlovitskaia et al. and/or Ritter II et al. references, remedy the deficiencies of the references discussed above. Diatlovitskaia, as previously noted, is cited simply due to its disclosure that the

ganglioside GD3 expressed by breast carcinomas, which is an example of a cancer of epithelial origin (see, e.g., page 11, lines 3-4 of the Office Action) whereas Ritter II is cited for its disclosure that GD3 derivatives such as GD3 lactone are more immunogenic than GD3 (see Office Action, page 11, lines 5-6). These disclosures do not supply the aspects of the invention missing from the above-discussed references. Applicants thus submit that the claimed invention is patentable over all of the cited references and respectfully request the Examiner to reconsider and withdraw the rejections of the claims under 35 U.S.C. §103(a).

NEW GROUNDS OF REJECTION

In ¶12 on page 12 of the Office Action claims 126-128 and 146 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement.

In particular, the Examiner stated that claims 126-128 recite ranges that are not described in the specification. Claims 126-128 have been cancelled in this Amendment and thus the §112 rejection of those claims is moot.

The Examiner stated that claim 146 is drawn to a method for delaying recurrence of melanoma. The Examiner stated that there does not appear to be support in the specification for methods for delaying recurrence of melanoma. The Examiner stated that the passages pointed to by applicant as providing support do not teach the recited references and do not teach methods for delaying the recurrence of melanoma. In response, claim 146 has been cancelled and thus the rejection of claim 146 under §112 also is moot.

SUMMARY

For the reasons set forth, applicants respectfully request that the Examiner reconsider and withdraw the various grounds of objection and rejection and earnestly solicit allowance of the now pending claims.

If a telephone interview would be of assistance in advancing prosecution of the subject application, applicants' attorneys invite the Examiner to telephone either of them at the number provided below.

A check for FIVE HUNDRED AND THIRTY DOLLARS ($530.00) is enclosed herewith. This amount has been determined by adding the fee of $475.00 due for the three-month extension of time for filing this response to the fee of $55.00 due for the filing of the Terminal Disclaimer ($475.00 + $55.00 = $530.00). If any additional fees are required, authorization is hereby given to charge the amount of such required fee(s) to Deposit Account No. 03-3125.

Respectfully submitted,

John P. White
Registration No. 28,678
Mark A. Farley
Registration No. 33,170
Attorneys for Applicant(s)
Cooper & Dunham, LLP
1185 Avenue of the Americas
New York, New York 10036
(212) 278-0400

I hereby certify that this correspondence is being deposited this date with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450

12-10-03
John P. White Date
Reg. No. 28,678
Mark A. Farley
Reg. No. 33,170

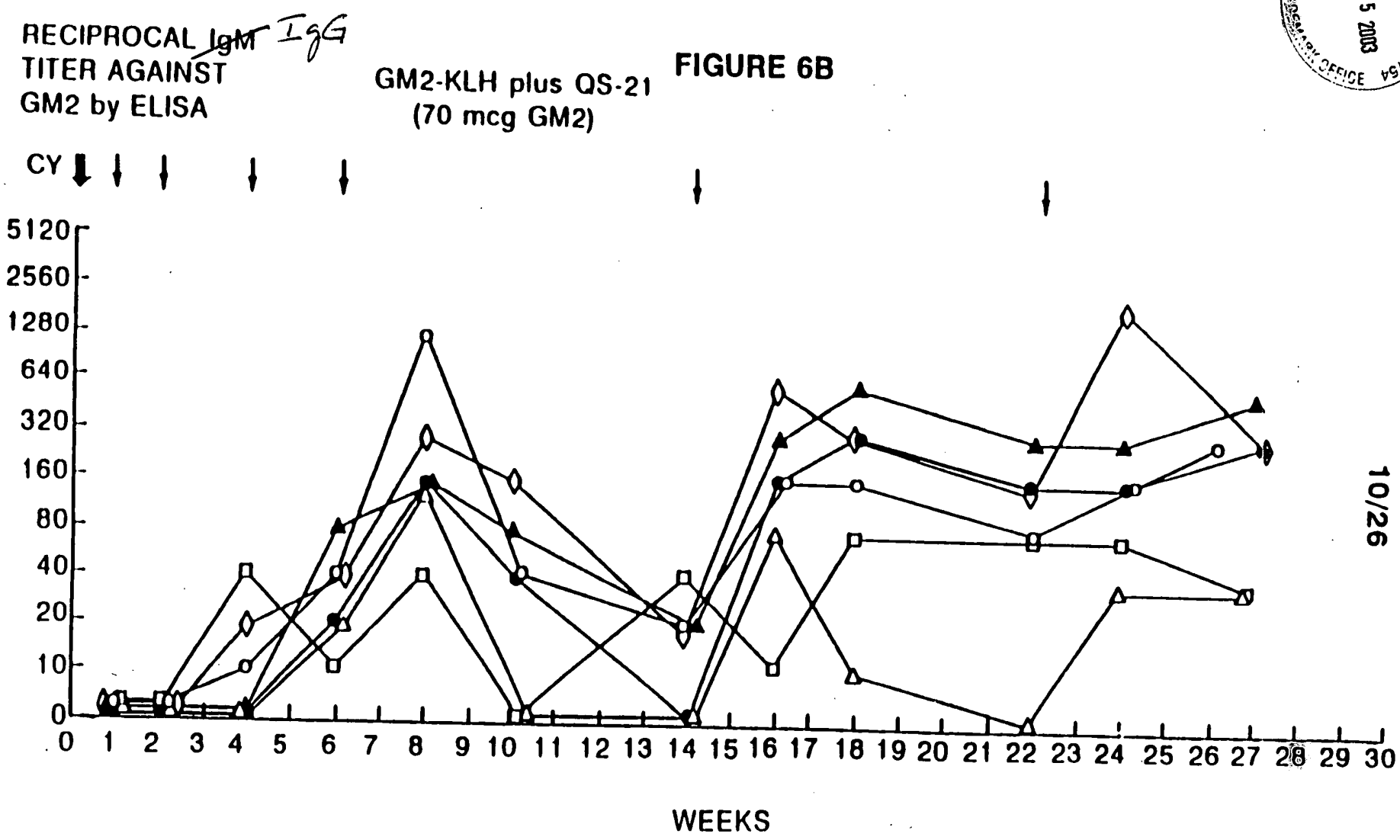
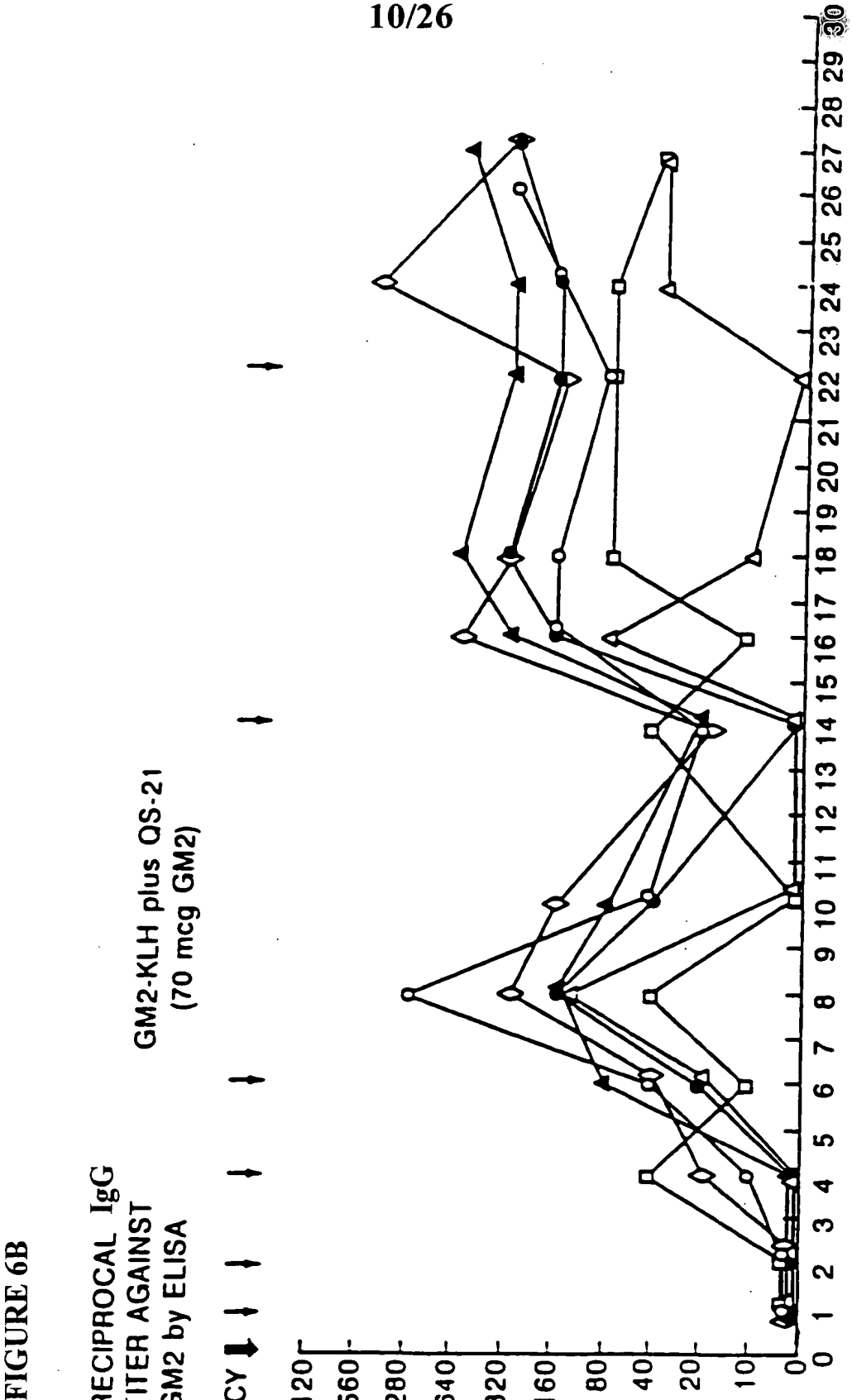


FIGURE 6B

10/26

"Annotated Marked-up Drawings"

FIGURE 6B



Dkt. 43016-D/JPW/MAF

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Philip Livingston and Friedhelm Helling

U.S. Serial No.: 08/477,147 Group Unit: 1645

Filed : June 7, 1995 Examiner: P. Duffy

For : GANGLIOSIDE-KLH CONJUGATE VACCINE PLUS QS-21

1185 Avenue of the Americas
New York, New York 10036

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SIR:

TERMINAL DISCLAIMER

Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021 is the Assignee of record of the entire right, title and interest in and to this application by virtue of an Assignment from Philip O. Livingston and Friedhelm Helling to Sloan-Kettering Institute for Cancer Research which was recorded in the U.S. Patent and Trademark Office on August 14, 1995 at Reel No. 7580, Frame No. 0708, a copy of which is attached hereto as **Exhibit 1**.

Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021 is also the Assignee of record of the entire right, title and interest in and to U.S. Serial No. 08/475,784 filed June 7, 1995, by virtue of an Assignment from Philip O. Livingston and Friedhelm Helling to Sloan-Kettering Institute for Cancer Research which was recorded in the U.S. Patent and Trademark Office on November 13, 1995 at Reel No. 7742, Frame 0338, a copy of which is attached hereto as **Exhibit 2**.

Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021 is additionally the Assignee of record of the entire right, title and interest in and to U.S. Serial No. 08/477,097 filed June 7, 1995 by virtue of an Assignment from Philip O. Livingston and Friedhelm Helling to Sloan-Kettering Institute for Cancer Research which was recorded in the U.S. Patent and Trademark Office on November 9, 1995 at Reel No. 7707, Frame No. 0968, a copy of which is attached hereto as **Exhibit 3**.

Sloan-Kettering Institute for Cancer Research hereby disclaims, except as provided below, the terminal portion of the statutory term of any patent granted on the above-identified application which would extend beyond the earliest expiration date of the full statutory term, as defined in 35 U.S.C. §154 to §156 and §173, of any patent issuing from U.S. Serial No. 08/475,784 and any patent issuing from U.S. Serial No. 08/477,097 and hereby agrees that any patent issued from the present application shall be enforceable only for and during such period that the legal title to such patent shall be the same as the legal title to both any patent issuing from U.S. Serial No. 08/475,784 and to any patent issuing from U.S. Serial No. 08/477,097, this agreement to run with any patent granted on the present application and to be binding upon the grantor, its successors and assignees.

In making the above disclaimer Sloan-Kettering Institute for Cancer Research does not disclaim the terminal part of any patent granted on the present application that would extend to the earliest expiration date of the full statutory term, as defined in 35 U.S.C. §154 to §156 and §173, of any patent issuing from U.S. Serial No. 08/475,784 and any patent issuing from U.S. Serial No. 08/477,097 in the event that either or both any patent issuing from U.S.

Serial No. 08/475,784 and any patent issuing from U.S. Serial No. 08/477,097 later: expires for failure to pay a maintenance fee, is found unenforceable, is found invalid by a court of competent jurisdiction, is statutorily disclaimed in whole or terminally disclaimed under 37 C.F.R. §1.321, has all claims canceled by a reexamination certificate, is reissued, or is in any manner terminated prior to the expiration of its full statutory term.

I have reviewed the assignment attached hereto as **Exhibit 1** and certify that, to the best of my knowledge and belief, Sloan-Kettering Institute for Cancer Research has all right, title, and interest in and to the present application. I further certify that I am authorized to sign on behalf of the assignee, Sloan-Kettering Institute for Cancer Research.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issued thereon.

Date: 12/03/03

Sloan-Kettering Institute
for Cancer Research

By: James S. Quirk

James S. Quirk
Senior Vice-President,
Research Resources Management
Sloan-Kettering Institute
for Cancer Research

SPE/APC/MISC

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DECEMBER 18, 1995

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COOPER & DUNHAM, LLP
JOHN P. WHITE
1185 AVENUE OF THE AMERICAS
NEW YORK, NY 10036

UNITED STATES PATENT AND TRADEMARK OFFICE
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THE ENCLOSED DOCUMENT HAS BEEN RECORDED BY THE ASSIGNMENT DIVISION OF THE U.S. PATENT AND TRADEMARK OFFICE. A COMPLETE MICROFILM COPY IS AVAILABLE AT THE ASSIGNMENT SEARCH ROOM ON THE REEL AND FRAME NUMBER REFERENCED BELOW.

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RECORDATION DATE: 08/14/1995

REEL/FRAME: 7580/0708
NUMBER OF PAGES: 4

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
LIVINGSTON, PHILIP O.

DOC DATE: 06/30/1995

ASSIGNOR:
HELLING, FRIEDHELM

DOC DATE: 07/25/1995

ASSIGNEE:
SLOAN-KETTERING INSTIUTE FOR CANCER RESEARCH
1275 YORK AVNEUE
NEW YORK, NEW YORK 10021

SERIAL NUMBER: 08477147
PATENT NUMBER:

FILING DATE: 06/07/1995
ISSUE DATE:

KEITH GOODE, EXAMINER
ASSIGNMENT DIVISION
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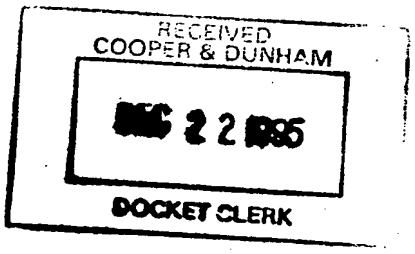

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100050002

Docket No. 43016-D

FORM PTO-1595 (Substitute) (Rev. 6-93)

U.S. DEPARTMENT OF COMMERCE
Patent and Trademark Office

To the Honorable Commissioner of Patents and ... ginal documents or copy thereof.

1. Name of conveying party(ies):
Philip O. Livingston and Friedhelm Helling

Additional name(s) of conveying party(ies) attached?
☐ Yes ☒ No

2. Name and address of receiving party(ies):
Name: Sloan-Kettering Institute for Cancer Research
Internal Address:
Street Address: 1275 York Avenue
City/State/Zip: New York, New York 10021

Additional name(s) & address(es) attached?
☐ Yes ☒ No

3. Nature of Conveyance:
☒ Assignment ☐ Merger
☐ Security Agreement ☐ Change of Name
☐ Other
Execution Date: July 25, 1995 & June 30, 1995

4. Application number(s) or patent number(s): If this document is being filed together with a new application, the execution date of the application is:

A. Patent Application No.(s)
U.S. Serial No. 08/477,147
Filed June 7, 1995

B. Patent No.(s)

Additional numbers attached? ☐ Yes ☒ No

5. Name and address of party to whom correspondence concerning document should be mailed:
Name: John P. White
Internal Address:
Street Address: Cooper & Dunham LLP
1185 Avenue of the Americas
City/State/Zip: New York, New York 10036

6. Total number of applications and patents involved: 1

7. Total fee (37 CFR 3.41):..... $ 40.00
☐ Enclosed
☒ Authorized to be charged to deposit account

8. Deposit account number:
03-3125
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W11392 08/21/95 08477147 03-3125 100 581 40.00CH

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9. Statement and signature.
To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

Albert Wai-Kit Chan — Name of Person Signing
Albert Wai Kit Chan — Signature
8/11/95 — Date

I hereby certify that this paper is being deposited this date with the U.S. Postal Service as first class mail addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Albert Wai-Kit Chan 8/11/95
Albert Wai-Kit Chan Date
Reg. No. 36,479

Total number of pages including cover sheet, attachments and document: 4

OMB No. 0651-0011 (exp. 6/94)

Mail documents to be recorded with required cover sheet information to:
Commissioner of Patents and Trademarks, Box Assignments
Washington, D.C. 20231

Assignment

In consideration of One Dollar ($1.00), and other good and valuable considerations, the receipt of which is hereby acknowledged, we, the undersigned,

Philip O. Livingston and Friedhelm Helling residing at 156 East 79th Street, New York, New York, 10021 and 504 East 63rd Street, Apartment 25N, New York, New York 10021 respectively,

Hereby sell, assign and transfer to Sloan-Kettering Institute for Cancer Research a corporation of the State of New York having a place of business at 1275 York Avenue in the County of New York and State of New York its successors, assigns and legal representatives, the entire right, title and interest for all countries, in and to any and all inventions which are disclosed and claimed, and any and all inventions which are disclosed but not claimed, in the application for United States Patent, which has been executed by the undersigned on July 25, 1995 and June 30, 1995 and is entitled

GANGLIOSIDE-KLH CONJUGATE VACCINE PLUS QS-21

(U.S. Serial No. 08/477,147, filed June 7, 1995, a continuation of PCT International Application No. PCT/US94/00757, filed January 21, 1994, which is a continuation of U.S. Serial No. 08/009,268, filed January 22, 1993)

and in and to said application and all divisional, continuing, substitute, renewal, reissue, and all other applications for U.S. Letters Patent or other related property rights in any and all foreign countries which have been or shall be filed on any of said inventions disclosed in said application; and in and to all original and reissued patents or related foreign documents which have been or shall be issued on said inventions;

Authorize and request the Commissioner of Patents of the United States to issue to said Assignee, the corporation above named, its successors, assigns and legal representatives, in accordance with this assignment, any and all United States Letters Patent on said inventions or any of them disclosed in said application;

Agree that said Assignee may apply for and receive foreign Letters Patent or rights of any other kind for said inventions, or any of them; and may claim, in applications for said foreign Letters Patent or other rights, the priority of the aforesaid United States patent application under the provisions of the International Convention of 1883 and later modifications thereof, under the Patent Cooperation Treaty, under the European Patent Convention or under any other available international agreement; and that, when requested, without charge to, but at the expense of, said Assignee, its successors, assigns and legal representatives, to carry out in good faith the intent and purpose of this assignment, the undersigned or the undersigned's executors or administrators will, for the United States and all foreign countries, execute all divisional, continuing, substitute, renewal, reissue, and all other patent applications or other documents on any and all said inventions; execute all rightful oaths, assignments, powers of attorney and other papers; communicate to said Assignee, its successors, assigns and representatives, all facts known and documents available to the undersigned relating to said inventions and the history thereof; testify in all legal proceedings; and generally do everything possible which said Assignee, its successors, assigns or representatives shall consider desirable for aiding in securing, maintaining and enforcing proper patent protection for said inventions and for vesting title to said inventions and all applications for patents or related foreign rights and all patents on said inventions, in said Assignee, its successors, assigns and legal representatives; and

Covenant with said Assignee, its successors, assigns and legal representatives that no assignment, grant, mortgage, license or other agreement affecting the rights and property herein conveyed has been made to others by the undersigned, and that full right to convey the same as herein expressed is possessed by the undersigned.

Date: 6/30/95 19

Philip G. Livingston [L.S.]

Witness: Vera Hinck

Vera Hinck

45 Brooklyn Avenue

West Hempstead NY

11552

Date: _____ 19

_____ [L.S.]
Friedhelm Helling

Witness: _____

_____

_____

_____

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Date: _____ 19

Witness: _____

_____

_____

_____

_____ [L.S.]
Philip O. Livingston

Date: 7/25 1995

Witness: Monica Bocchia
MONICA BOCCHIA
504 E 63rd ST. APT 25N NEW YORK, NY, 10021

Friedhelm Helling [L.S.]
Friedhelm Helling

ASSIGNMENT BY

Philip O. Livingston and Friedhelm Helling,

TO

Sloan-Kettering Institute for Cancer Research,

Application for Lettere Patent of the United States for GANGLIOSIDE-KLH CONJUGATE VACCINE PLUS QS-21

Attorneys for John P. White

Cooper & Dunham LLP
1185 Avenue of the Americas
New York, N.Y. 10036
212-278-0400

AKC

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
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Washington, D.C. 20231

APRIL 07, 1996

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COOPER & DUNHAM LLP
ALBERT WAI-KIT CHAN
1185 AVENUE OF THE AMERICAS
NEW YORK, NY 10036

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RECORDATION DATE: 11/13/1995

REEL/FRAME: 7742/0338
NUMBER OF PAGES: 4

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
LIVINGTON, PHILIP O.

DOC DATE: 07/25/1995

ASSIGNOR:
HELLING, FRIEDHELM

DOC DATE: 06/30/1995

ASSIGNEE:
SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH
1275 YORK AVENUE
NEW YORK, NEW YORK 10021

SERIAL NUMBER: 08475784
PATENT NUMBER:

FILING DATE: 06/07/1995
ISSUE DATE:

DOROTHY RILEY, EXAMINER
ASSIGNMENT DIVISION
OFFICE OF PUBLIC RECORDS

12-29-1995

No. 43016-C

FORM PTO-1595 (Substitute) (Rev. 6-93) RECOR

U.S. DEPARTMENT OF COMMERCE tent and Trademark Office

To the Honorable Commissioner of Patents and Trade documents or copy th reof.

100111931

1. Name of conveying party(ies): Livingston and Friedhelm Helling

Addi name(s) of conveying party(ies) attached? ☐ Yes ☒ No

Receiving party(ies):

Name: Sloan-Kettering Institute for Cancer Research

Internal Address:

Street Address: 1275 York Avenue

City/State/Zip: New York, New York 10021

Additional name(s) & address(es) attached? ☐ Yes ☒ No

3. Nature of Conveyance:

☒ Assignment ☐ Merger

☐ Security Agreement ☐ Change of Name

☐ Other

Execution Date: July 25, 1995 and June 30, 1995

4. Application number(s) or patent number(s): If this document is being filed together with a new application, the execution date of the application is:

A. Patent Application No.(s)

U.S. Serial No. 08/475,784
Filed June 7, 1995

B. Patent No.(s)

Additional numbers attached? ☐ Yes ☒ No

5. Name and address of party to whom correspondence concerning document should be mailed:

Name: John P. White

Internal Address:

Street Address: Cooper & Dunham LLP

1185 Avenue of the Americas

City/State/Zip: New York, New York 10036

6. Total number of applications and patents involved: 1

7. Total fee (37 CFR 3.41):. . . . . $ 40.00

☐ Enclosed

☒ Authorized to be charged to deposit account

8. Deposit account number: 03-3125

(Attach duplicate copy of this page if paying by deposit account)

WT10053 11/29/95 08475784 03-3125 100 581 40.00CH

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9. Statement and signature.

To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.

Albert Wai-Kit Chan — Albert Wai Kit Chan — 11/9/95

Name of Person Signing — Signature — Date

I hereby certify that this paper is being deposited this date with the U.S. Postal Service as first class mail addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Albert Wai Kit Chan 11/9/95

Albert Wai-Kit Chan Date

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OMB No. 0651-0011 (exp. 4/94)

Mail documents to be recorded with required cover sheet information t : Commissioner of Patents and Trademarks, Box Assignments Washington, D.C. 20231

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(U.S. Serial No. 08/475,784 , filed June 7, 1995, a continuation of PCT International Application No. PCT/US94/00757, filed January 21, 1994, which is a continuation of U.S. Serial No. 08/009,268, filed January 22, 1993)

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Philip O. Livingston [L.S.]
Philip O. Livingston

Date: 6/30/95 19

Witness: Vera Hinck
Vera Hinck
45 Brooklyn Avenue
West Hempstead, NY 11552

[L.S.]
Friedhelm Helling

Date: 19

Witness:

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_____ [L.S.]
Philip O. Livingston

Date: _____ 19

Witness: _____

_____

_____

_____

Friedhelm Helling [L.S.]

Date: 7.25. 1995

Witness: Monica Bocchia

MONICA BOCCHIA

504 E 63rd ST. APT 25N NEW YORK, NY, 10021

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MARCH 25, 1996

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JOHN P. WHITE
COOPER & DUNHAM L.L.P.
1185 AVENUE OF THE AMERICAS
NEW YORK, NY 10036

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RECORDATION DATE: 11/09/1995
REEL/FRAME: 7707/0968
NUMBER OF PAGES: 4

BRIEF: ASSIGNMENT OF ASSIGNOR'S INTEREST (SEE DOCUMENT FOR DETAILS).

ASSIGNOR:
LIVINGSTON, PHILIP O.
DOC DATE: 06/30/1995

ASSIGNOR:
HELLING, FRIEDHELM
DOC DATE: 07/25/1995

ASSIGNEE:
SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH
1275 YORK AVENUE
NEW YORK, NEW YORK 10021

SERIAL NUMBER: 08477097
FILING DATE: 06/07/1995
PATENT NUMBER:
ISSUE DATE:

JERYL MCDOWELL, EXAMINER
ASSIGNMENT DIVISION
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12-01-1995

Docket No. 43016-B

FORM PTO-1595 (Substitute)

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Patent and Trademark Office

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1. Name of conveying party(ies):
Philip O. Livingston and Friedhelm Helling

Additional name(s) of conveying party(ies) attached?
☐ Yes ☒ No

2. Name and address of receiving party(ies):
Name: Sloan-Kettering Institute for Cancer Research
Internal Address:
Street Address: 1275 York Avenue
City/State/Zip: New York, New York 10021

Additional name(s) & address(es) attached?
☐ Yes ☒ No

3. Nature of Conveyance:
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Execution Date: July 25, 1995 and June 30, 1995

4. Application number(s) or patent number(s): If this document is being filed together with a new application, the execution date of the application is:

A. Patent Application No.(s)
Serial No. 08/477,097 filed June 7, 1995

B. Patent No.(s)

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Name: John P. White
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Albert Wai-Kit Chan — Name of Person Signing
Albert Wai-Kit Chan — Signature
11/7/95 — Date

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Albert Wai-Kit Chan Date
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Date: 6/30/95 19

Philip J. Livingston [L.S.]

Witness: Vera Hinck

Vera Hinck

45 Brooklyn Avenue

West Hempstead NY 11552

Date: _____ 19

Friedhelm Helling [L.S.]

Witness: _____

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_____ [L.S.]
Philip O. Livingston

Date: _____ 19

Witness: _____

_____

_____

_____

Friedhelm Helling [L.S.]

Date: 7.25. 1995

Witness: Monica Bocchia

MONICA BOCCHIA

504 E 63rd STREET APT 25N.

NEW YORK NY, 10021

Induction of Antibodies against GM2 Ganglioside by Immunizing Melanoma Patients Using GM2-Keyhole Limpet Hemocyanin + QS21 Vaccine: A Dose-Response Study[1]

Paul B. Chapman,[2] D. M. Morrissey, K. S. Panageas, W. B. Hamilton, C. Zhan, A. N. Destro, L. Williams, R. J. Israel, and P. O. Livingston[3]

Department of Medicine, Clinical Immunology Service, [P. B. C., A. N. D., L. W., P. O. L.], and Department of Epidemiology and Biostatistics [K. S. P.], Memorial Sloan-Kettering Cancer Center, New York, New York 10021, and Progenics Pharmaceuticals, Inc., Tarrytown, New York [D. M. M., W. B.H., C. Z., R. J. I.]

ABSTRACT

In a previous randomized Phase III trial (P. O. Livingston *et al.*, J. Clin. Oncol., *12:* 1036–1044, 1994), we demonstrated that immunization with GM2 and *bacille Calmette-Guérin* reduced the risk of relapse in stage III melanoma patients who were free of disease after surgical resection and who had no preexisting anti-GM2 antibodies. That vaccine formulation induced IgM anti-GM2 antibodies in 74% but induced IgG anti-GM2 antibodies in only 10% of the patients. To optimize the immune response against GM2, a reformulated vaccine was produced conjugating GM2 to keyhole limpet hemocyanin (KLH) and using the adjuvant QS21 (GM2-KLH/QS21). In pilot studies, 70 μg of vaccine induced IgG anti-GM2 antibodies in 76% of the patients. We wished to define the lowest vaccine dose that induced consistent, high-titer IgM and IgG antibodies against GM2. Fifty-two melanoma patients who were free of disease after resection but at high risk for relapse were immunized with GM2-KLH/QS21 vaccine at GM2 doses of 1, 3, 10, 30, or 70 μg on weeks 1, 2, 3, 4, 12, 24, and 36. Serum collected at frequent and defined intervals was tested for anti-GM2 antibodies. Overall, 88% of the patients developed IgM anti-GM2 antibodies; 71% also developed IgG anti-GM2 antibodies. GM2-KLH doses of 3–70 μg seemed to be equivalent in terms of peak titers and induction of anti-GM2 antibodies. At the 30-μg dose level, 50% of the patients developed complement fixing anti-GM2 antibodies detectable at a serum dilution of 1:10. We conclude that the GM2-KLH/QS21 formulation is more immunogenic than our previous formulation and that 3 μg is the lowest dose that induces consistent, high-titer IgM and IgG antibodies against GM2.

INTRODUCTION

GM2 is a ganglioside expressed on the surface of most melanomas and has been demonstrated to be immunogenic (1, 2). In our previous studies, we demonstrated that melanoma patients who were free of disease after complete surgical resection and who have natural or vaccine-induced antibodies to GM2 have a decreased risk of relapse (3). Immunization with GM2 alone does not induce antibodies (4); induction of optimal immunity against GM2 requires immunization with a potent adjuvant (5). In previous trials, GM2 was mixed with *bacille Calmette-Guérin*, which resulted in short-lived IgM antibodies (titers ≥ 1:80) in approximately 74% of patients, but rarely induced IgG antibodies against GM2 (approximately 10% of patients immunized; Ref. 3). Although IgM antibodies are potent mediators of CMC,[4] we hypothesized that the additional induction of an IgG response against GM2 could result in a more pronounced clinical effect. However, induction of IgG antibodies against carbohydrate antigens such as gangliosides would require a T_H epitope to provide the appropriate signals for immunoglobulin class switching.

To address this issue, GM2 was conjugated to KLH, a carrier protein known to provide T-cell help and administered with adjuvant QS21, a saponin fraction extracted from the bark of the South American tree *Quillaja saponaria Molina* (6). In two pilot studies using GM2 doses of 70 μg, this formulation resulted in high-titer IgG antibodies against GM2 (5, 7). Both IgM and IgG antibodies reacted with $GM2^+$ tumor cells by flow cytometry and induced complement-mediated lysis (8). In these two trials, 32 (76%) of 42 patients developed IgG antibodies against GM2 at titers ≥1:80 when doses of QS21 ≥100 μg were used. Thus, IgG antibodies could consistently be induced against GM2.

The objective of the current trial was to determine the minimal dose of GM2-KLH required for a consistent, high-titer IgM and IgG antibody response. This is one of the first dose-response studies carried out in patients receiving a defined cancer vaccine and identifies a dose that is appropriate for future Phase III trials.

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[1] Supported by National Cancer Institute Grant PO1 CA33049.
[2] To whom requests for reprints should be addressed, at Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021. Phone: (212) 639-5015; Fax: (212) 794-4352.
[3] P. O. L. is a paid consultant and a shareholder in Progenics Pharmaceuticals.

[4] The abbreviations used are: CMC, complement-mediated cytotoxicity; KLH, keyhole limpet hemocyanin; AUC, area under curve, LDH; lactate dehydrogenase.

Table 1 Dose levels and formulations of GM2-KLH + QS21 vaccine

Dose level (μg of GM2)	No. of patients immunized
1	5
3	10
10	10
30	20[a]
70	7
Total	52

[a] The second 10 patients at the 30-μg dose level received vaccine in which the GM2-KLH and QS21 were vialed separately and mixed just prior to administration.

Table 2 Patient characteristics of 52 patients treated

Stage	
II (>4 mm)	4
III	39
IV	9
Gender	
Male/Female	34/18
Primary site	
Trunk	24
Extremity	20
Head/neck	6
Unknown	2
Median age (range)	60 (26–77)
Median time in months from complete resection until first vaccine (range)	5.7 (2.1–12)

MATERIALS AND METHODS

Vaccine Preparation

GM2-KLH was prepared with GM2 from bovine brain and supplied by Progenics Pharmaceuticals, Inc. (Tarrytown, New York) as described previously (5, 9). QS21 was supplied by Aquila BioPharmaceuticals (Framingham, MA).

In general, the vaccine was formulated in a single vial containing both GM2-KLH and QS21. However, a group of 10 patients immunized at the 30-μg dose level were immunized with GM2-KLH and QS21 vialed separately. For these patients, the GM2-KLH and QS21 were mixed by the pharmacist just prior to administration.

Patient Eligibility

Melanoma patients with American Joint Committee on Cancer stage III or IV, or deep stage II (>4 mm), who were free of disease after complete surgical resection were eligible. All of the pathology was confirmed by the Memorial Hospital Pathology Department. In general, patients were started on vaccine within 10 months of surgical resection, but patients were still eligible even after 10 months if their risk of relapse was felt to be >50%. All of the patients signed written informed consent.

Patients were excluded if their Karnofsky performance status was <80, if they had received systemic therapy or radiotherapy within the previous 8 weeks, or if they had a medical condition that would make it difficult to complete the full course of vaccination or to respond immunologically to the vaccine. Women who were pregnant or breast-feeding were not eligible.

Treatment Plan

This trial was carried out under an IND from the United States Food and Drug Administration. Within 4 weeks of starting vaccinations, patients had a physical exam, chest X-ray or chest CT, complete blood count, and comprehensive chemistry screen. An electrocardiogram was required within 10 months of starting the study.

Vaccines were administered by the Clinical Immunology nurses (Clinical Immunology Service, Memorial Sloan-Kettering Cancer Center) as a s.c. injection (final volume, 0.75 ml). Vaccinations were administered on weeks 1, 2, 3, 4, 12, 24, and 36.

This study was designed to compare the immunological effects of different doses of GM2-KLH vaccine. Groups of 5–10 patients were accrued to each of five vaccine dose levels in which the GM2-KLH concentration was adjusted to deliver a GM2 dose of 1, 3, 10, 30, or 70 μg (Table 1). All of the vaccinations contained 100 μg of QS21. Subsequently, the vaccine formulation was changed so that the GM2-KLH and QS21 were prepared in separate vials and mixed just prior to vaccine administration. Using this "two-vial system," an additional 10 patients were immunized at the 30-μg dose level.

Treatment Evaluation

Serological Analysis. Serum was collected immediately prior to each vaccination (including pretreatment), and on weeks 6, 13, 18, 26, 30, 38, and 42. In addition, serum was collected 3 and 6 months after the 7th and final vaccination. Anti-GM2 antibodies were measured using an ELISA method in which GM2 ganglioside is adsorbed to 96-well polystyrene microtiter plates. The remaining binding sites on the plate were blocked by PBS/casein/Tween 20 buffer. Serially diluted patient sera or controls were added, and bound antibody was detected using a goat antihuman IgM or IgG antibody (heavy-chain-specific) conjugated to alkaline phosphatase. Plates were developed using *p*-nitrophenyl phenol substrate, and absorbance was read at 405 nm with a correction of 620 nm. Antibody titer was defined as the highest dilution of patient serum yielding a corrected absorbance of 0.1. Pooled human serum from previously vaccinated patients with a known anti-GM2 antibody titer or pooled normal human serum with no anti-GM2 reactivity were used as positive and negative controls, respectively. A positive serological response was defined as an anti-GM2 titer ≥ 1:80 observed at two or more time points.

The antibody titers plotted *versus* time were also analyzed as the AUC using Prism version 2.01 software (Graph Pad Software, Inc., San Diego, CA). The AUC of the antibody response was considered to represent the overall exposure to anti-GM2 IgG or IgM over time.

CMC Assay. CMC assays were performed by the LDH release method (Boehringer-Mannheim). SK-MEL31 (GM2-positive) or SK-MEL24 (GM2-negative) were plated in 96-well tissue culture plates and incubated at 37°C in a humidified CO_2 incubator. The medium was removed, and plain DMEM containing human serum complement standard (Sigma Chemical Co., St. Louis, MO) was added along with the pre- or postim-

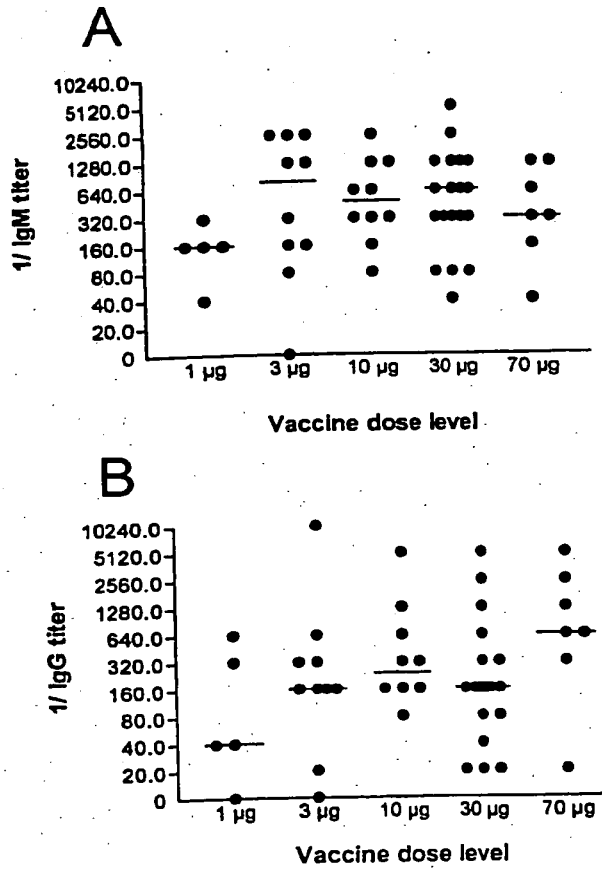

Fig. 1 Peak anti-GM2 antibody titers in patients immunized with GM2-KLH + QS21 at GM2 doses of 1, 3, 10, 30, or 70 µg. *Each dot*, a single patient. The *horizontal lines*, the median peak titers for each dose level. *A*, peak IgM titers; *B*, peak IgG titers.

munization serum to be tested in duplicate wells. The postimmunization serum tested was the serum sample showing the highest IgM anti-GM2 titers for each patient. Both the complement and serum were used at a final dilution of 1:10. In positive control wells, 1% NP40 was added to measure maximal release. The plate was returned to the incubator for 16 h. The supernatants were removed and transferred to a 96-well ELISA plate for analysis. LDH substrate/catalyst was added, and the plate was incubated in the dark at 25°C for 20 min. The plate was read on a spectrophotometer at 492 nm. Each patient's preimmune CMC reading served as the control for the postimmune CMC result. Percent-specific lysis against each cell line was calculated as follows:

$$\frac{\text{(Postimmune serum LDH release} - \text{preimmune serum LDH release)}}{\text{NP40 LDH release}}$$

Clinical Evaluation. Patients were evaluated clinically at Memorial Hospital on weeks 12, 24, and 36 and on three months after the 7th vaccination. A chest X-ray, complete blood count, and comprehensive screening profile were repeated at the time of the 5th and 7th vaccination; an electrocardiogram was repeated at the time of the 7th vaccination. Toxicity was scored using standard criteria (10).

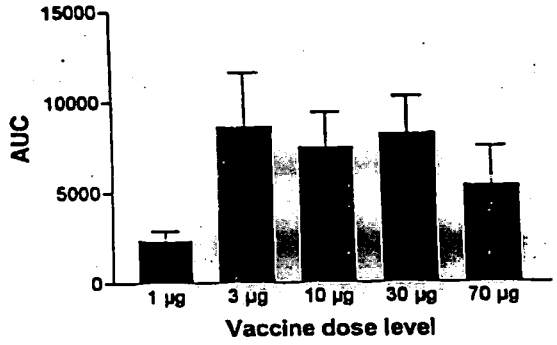

Fig. 2 AUC analysis for the IgM anti-GM2 responses at each dose level. The AUC was calculated for each patient up to week 30. *Height of the columns*, the mean (± SE) AUC for each dose level.

RESULTS

Patient Characteristics. Fifty-two patients were entered on this trial between January 1995 and April 1996 (Table 2). There were 34 men and 18 women. Most (75%) of the patients had stage III melanoma; 8% had deep stage II, and 17% had stage IV. The patients had been free of disease for a median of 5.7 months before beginning the trial.

Serological Results. Applying rigorous definitions of response (defined in "Materials and Methods") 88% of the patients immunized in this study developed an IgM response against GM2; 71% developed an IgG response. Fig. 1 shows the peak anti-GM2 titers attained at each dose level. For IgM, the median peak titers ranged from 1:160 to 1:800; for IgG the median peak titers ranged from 1:40 to 1:640. When comparing the incidence of nonresponding patients (peak titers ≤1:40) for IgM and IgG at each of the dose levels, we found no difference for the IgM response ($P = 0.73$; χ^2) or IgG response ($P = 0.19$; χ^2). From the exploratory analysis, it appeared that there were fewer IgG responses at the 1-µg dose level.

An AUC analysis was performed for both IgM and IgG anti-GM2 responses on each patient until week 30, and the mean AUCs at each dose level were compared. For the IgM anti-GM2 response, the mean AUC at the 1-µg dose level was lower than the mean AUC at any of the other dose levels (Fig. 2). The mean AUC for the IgG response was also lower in patients treated at the 1-µg dose level compared with the mean AUCs at the other dose levels (data not shown), but this difference was not statistically significant. There were no differences in the AUC for the other dose levels.

Given that the 1-µg dose level seemed to have a lower incidence of inducing IgG against GM2 and a lower mean AUC for the IgM response, we concluded that the 1-µg dose level was less immunogenic than the other dose levels. As a result, we focused on the 3-, 10-, 30-, and 70-µg dose levels.

Fig. 3 illustrates the median anti-GM2 IgM and IgG titers for patients immunized at the 3-, 10-, 30-, and 70-µg dose

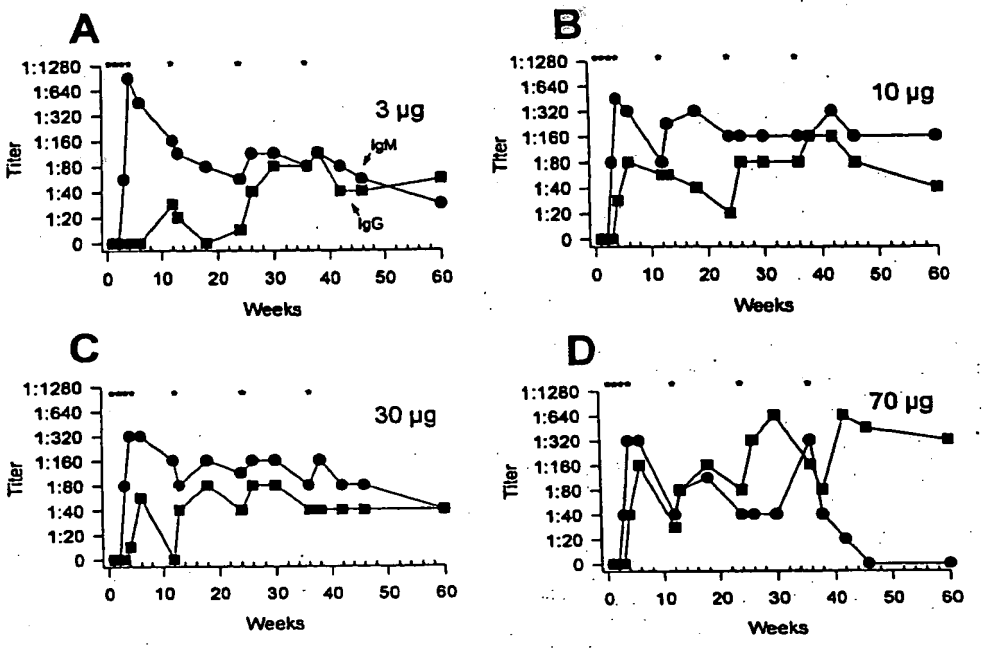

Fig. 3 Median anti-GM2 antibody titers in patients immunized with GM2-KLH + QS21 at GM2 doses of 3 μg (*A*), 10 μg (*B*), 30 μg (*C*), or 70 μg (*D*). IgM titers (●) and IgG titers (■) are shown separately at each dose level. *, administration of vaccine.

levels. At these four dose levels, there was a consistent IgM response followed by an IgG response. Both the IgM and IgG responses were sustained for months after the final immunization. At week 60 (5½ months after the last immunization), serum was available on 20 patients who had developed an IgM response and 19 patients who had developed an IgG response. Analysis of these sera showed that the IgM response persisted in 45% of the cases; the IgG response persisted in 53% of the cases (data not shown). This demonstrates that, in one-half of the patients who developed anti-GM2 antibodies, the antibody response persisted for at least 5½ months.

Most of the patients immunized on this trial received vaccine that had been formulated in one vial (*i.e.*, GM2-KLH and QS21 were stored together). However, 10 of the 20 patients immunized at the 30-μg dose level received vaccine formulated in two vials because we obtained evidence that the stability of the vaccine was enhanced if the GM2-KLH and QS21 were stored in separate vials and mixed just prior to vaccine administration. We compared the anti-GM2 response induced in patients immunized with the single-vial *versus* the two-vial formulation at the 30-μg dose level (Fig. 4). The median IgM titers were similar in the two groups; the median IgG titers were slightly lower in the group receiving vaccine formulated as two vials. All of the patients immunized with the single-vial formulation developed anti-GM2 antibodies, and only one patient immunized with the two-vial formulation failed to develop anti-GM2 antibodies. We conclude that there was no difference in the immunogenicity between the one-vial and the two-vial formulations.

CMC. Sera from 18 of the 20 patients treated at the 30-μg dose level were available to be tested for the ability to bind melanoma cells and to fix the complement (Fig. 5). In 9 of the 18 patients, the postvaccination sera showed an increase in

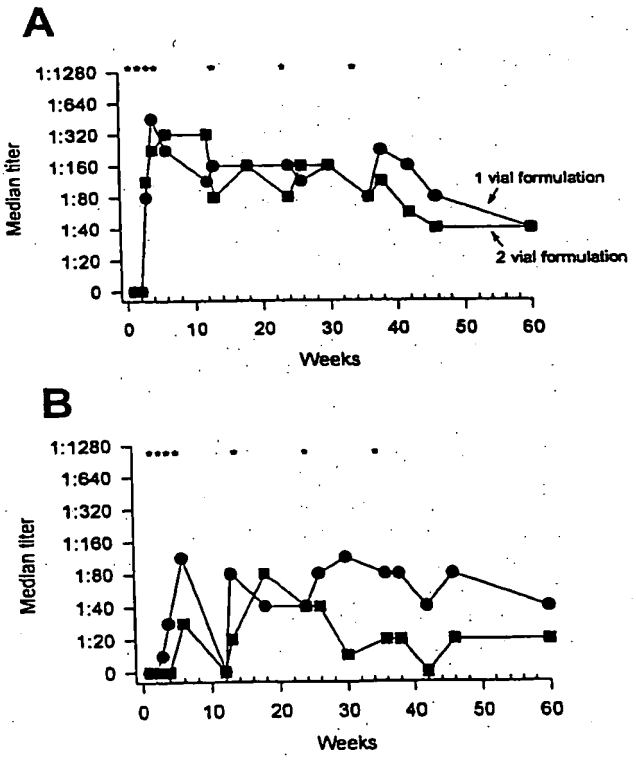

Fig. 4 Comparison of median anti-GM2 IgM titers (*A*) and IgG titers (*B*) among patients immunized at the 30-μg dose level. , patients immunized with vaccine formulated in a single vial; ■, patients immunized with vaccine formulated in 2 vials, in which the GM2-KLH and QS21 vialed separately; *, administration of vaccine.

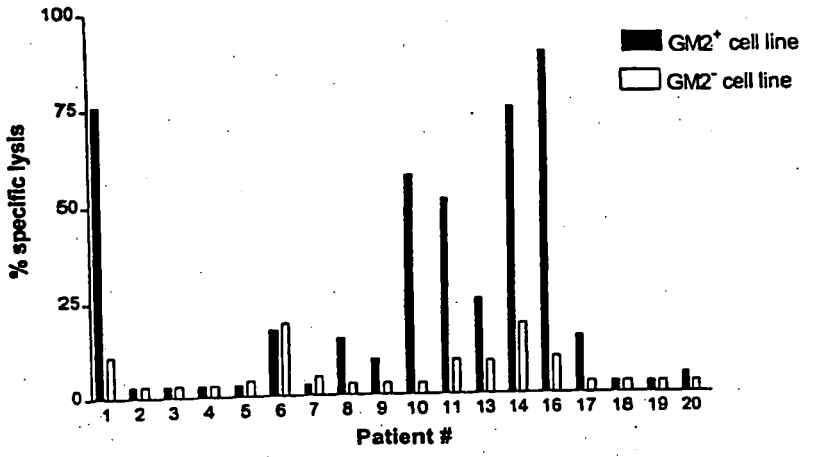

Fig. 5 CMC of sera from patients immunized at the 30-μg dose level. ■, the increase of CMC against a $GM2^+$ cell target in postvaccination sera compared with pretreatment sera. □, the increase of CMC against a $GM2^-$ cell target in postvaccination sera compared with pretreatment sera. Data for patients 12 and 15 are not available.

CMC compared to pretreatment that was specific for the $GM2^+$ cell target. In the remaining nine patients, there was either no increase in CMC compared with pretreatment levels (patients 2, 3, 4, 5, 7, 18, 19, and 20) or the increase was not specific for GM2 (patient 6). Induction of complement-fixing activity correlated with a peak IgM anti-GM2 titer of 1:640. All of the nine patients demonstrating CMC activity in their serum had peak IgM anti-GM2 titers ≥ 1:640 as opposed to only two of nine patients without CMC activity ($P = 0.002$; Fisher's exact test).

Toxicity. Virtually all of the patients experienced inflammation and/or pruritis at the site of injection attributed to the known effects of the QS21 adjuvant (7). Other common side effects were: (*a*) fever (71%); (*b*) mild fatigue (44%) and flu-like symptoms (58%); (*c*) chills (29%); and (*d*) myalgias (48%). These were self-limiting, never more severe than grade 2, and rarely lasted more than 24 h. Headache was seen in 66% of the patients and was grade 1–2 except in one patient with a grade-3 headache. These toxicities were felt to be due largely to QS21, which is consistent with the observation that there was no correlation between vaccine dose and toxicity. Grade 3 or 4 toxicity possibly related to vaccine occurred in four patients. One patient developed transient dyspnea, which resolved spontaneously. Another patient reported 2–3 days of severe dizziness, which also resolved spontaneously. One patient developed atrial flutter while on the study and required treatment. A fourth patient, with a history of migraine headaches, reported a grade 3 headache associated with vaccine therapy. No patient was taken off study because of toxicity.

DISCUSSION

The current trial confirms that vaccinating melanoma patients with GM2-KLH + QS21 induces both IgM and IgG antibodies against GM2. We observed that 88% of patients developed IgM anti-GM2 antibodies and 71% developed IgG anti-GM2 antibodies. This compares almost exactly with the immunological results observed in our previous pilot trials (5, 7). Because the previous trials used vaccine produced at Memorial Sloan-Kettering Cancer Center and the current trial used vaccine produced by Progenics Pharmaceuticals, Inc., this demonstrates that subsequent lots of the vaccine can be produced successfully and that the immunogenicity is reproducible. The results also show that the vaccine can be formulated either with QS21 or vialed separately and mixed with QS21 just prior to administration. We favor formulating GM2-KLH and QS21 in separate vials because of improved stability.

This is one of the first cancer vaccine trials to explore dose-response effects using a defined antigen. Our previous trials used GM2-KLH at a GM2 dose of 70 μg and demonstrated that all of the patients developed IgM anti-GM2 and 76% developed IgG anti-GM2. In this current trial, we have explored GM2 doses of 1, 3, 10, 30, and 70 μg. We conclude that the immunogenicity of GM2-KLH at a GM2 dose of 1 μg is suboptimal based on the fact that the 1-μg dose was less likely to induce IgG anti-GM2 antibodies. The mean AUC for the anti-GM2 IgM antibody responses was also lowest for the 1-μg dose level, which implies that this dose resulted in the lowest level of tumor-cell exposure to anti-GM2 antibody. At the higher vaccine doses (3, 10, 30, or 70 μg), there was no apparent difference in the immunogenicity of the vaccine. Peak titers, AUC, antibody responses over 60 weeks, and percent of nonresponding patients were similar at the 3-, 10-, 30-, and 70-μg dose levels.

In patients immunized at the 30-μg dose level, 50% of the patients developed antibodies that fixed complement and resulted in CMC against $GM2^+$ melanoma. CMC activity correlated with peak IgM anti-GM2 titers ≥1:640. This demonstrates that immunization induced anti-GM2 antibodies capable of binding cell-surface GM2 and mediating effector functions.

In at least one-half of the patients, the anti-GM2 antibody response persisted for more than 5½ months. This is consistent with the notion that the KLH carrier protein provides sufficient T-cell help to induce a more prolonged antibody response against GM2. It is also important to note that patients at the 70-μg dose level received a 23-fold higher KLH dose compared with patients at the 3-μg dose level, and that this was not associated with any excessive toxicity or decreased immunogenicity. This is reassuring as we consider construction of multivalent vaccines containing 4 or 5 antigens conjugated to KLH. Our results suggest that these higher

total KLH doses will neither be more toxic nor lead to diminished immunogenicity.

These studies provide a basis for additional trials with GM2-KLH + QS21. Future clinical trials will examine the effects of IFN-α on the anti-GM2 response induced by GM2-KLH + QS21, the immunogenicity of GM2-KLH + QS21 combined with GD2-KLH, and a Phase III trial comparing GM2-KLH + QS21 to IFN-α for the ability to prevent recurrence of melanoma in stage III patients. For these trials, a vaccine dose ≥3 μg of GM2 should be used.

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REFERENCES

1. Tai, T., Cahan, L. D., Tsuchida, T., Saxton, R. E., Irie, R. F., and Morton, D. L. Immunogenicity of melanoma-associated gangliosides in cancer patients. Int. J. Cancer, *35:* 607–612, 1985.

2. Livingston, P. O., Ritter, G., Oettgen, H. F., and Old, L. J. Immunization of melanoma patients with purified gangliosides. *In:* H. F. Oettgen (ed.), Gangliosides and Cancer, pp. 293–300. New York: VCH Publishers, Inc., 1989.

3. Livingston, P. O., Wong, G. Y. C., Adluri, S., Tao, Y., Padavan, M., Parente, R., Hanlon, C., Jones Calves, M., Helling, F., Ritter, G., Oettgen, H. F., and Old, L. J. Improved survival in stage III melanoma patients with GM2 antibodies: a randomized trial of adjuvant vaccination with GM2 ganglioside. J. Clin. Oncol., *12:* 1036–1044, 1994.

4. Livingston, P. O., Natoli, E. J., Jr., Calves, M. J., Stockert, E., Oettgen, H. F., and Old, L. J. Vaccines containing purified GM2 ganglioside elicit GM2 antibodies in melanoma patients. Proc. Natl. Acad. Sci. USA, *84:* 2911–2915, 1987.

5. Helling, F., Zhang, S., Shang, A., Adluri, S., Calves, M., Koganty, R., Longenecker, B. M., Yao, T-J., Oettgen, H. F., and Livingston, P. O. GM2-KLH conjugate vaccine: increased immunogenicity in melanoma patients after administration with immunological adjuvant QS-21. Cancer Res., *55:* 2783–2788, 1995.

6. Kensil, C. R., Patel, U., Lennick, M., and Marciani, D. Separation and characterization of saponins with adjuvant activity from *Quillaja saponaria Molina* cortex. J. Immunol., *146:* 431–437, 1991.

7. Livingston, P. O., Adluri, S., Helling, F., Yao, T. J., Kensil, C. R., Newman, M. J., and Marciani, D. Phase 1 trial of immunological adjuvant QS-21 with a GM2 ganglioside-keyhole limpet haemocyanin conjugate vaccine in patients with malignant melanoma. Vaccine, *12:* 1275–1280, 1994.

8. Livingston, P., Zhang, S., Adluri, S., Yao, T-J., Graeber, L., Ragupathi, G., Helling, F., and Fleisher, M. Tumor cell reactivity mediated by IgM antibodies *in sera* from melanoma patients vaccinated with GM2 ganglioside covalently linked to KLH is increased by IgG antibodies. Cancer Immunol. Immunother., *43:* 324–330, 1997.

9. Helling, F., Shang, A., Calves, M., Zhang, S., Ren, S., Yu, R. K., Oettgen, H. F., and Livingston, P. O. Increased immunogenicity of GD3 conjugate vaccines: comparison of various carrier proteins and selection of GD3-KLH for further testing. Cancer Res., *54:* 197–203, 1994.

10. Creekmore, S. P., Longo, D. L., and Urba, W. J. Principles of the clinical evaluation of biologic agents. *In:* V. T. J. DeVita, S. Hellman, and S. A. Rosenberg (eds.), Biologic Therapy of Cancer, pp. 67–86. Philadelphia: J. B. Lippincott Company, 1991.